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Piersma, Theunis; Van Der Velde, Marco

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Breeding season-specific sex diagnostics in the monomorphic House Martin *Delichon urbicum*

THEUNIS PIER SMA^{1,2*} and MARCO VAN DER VELDE¹

¹Animal Ecology Group, Centre for Ecological and Evolutionary Studies, University of Groningen, PO Box 14, 9750 AA Haren, The Netherlands and ²Department of Marine Ecology, Royal Netherlands Institute for Sea Research (NIOZ), PO Box 59, 1790 AB Den Burg, Texel, The Netherlands

Capsule Breeding adult females show a bare brood patch and a smaller cloacal protuberance than males.

Aims To examine the degree of sexual dimorphism in various phenotypic traits in House Martins.

Methods In the summers of 2005–07 in the northern Netherlands, 160 House Martins were captured. We measured wing length, keel length and body mass. The 115 adults were examined for the presence or absence of a bare brood patch, the colour of the belly plumage (greyish or pure white) and the diameter of the cloacal protuberance. A small blood sample was taken from the brachial vein and sex was assigned on the basis of a standard molecular assay on DNA from blood.

Results With regard to wing length and body mass, House Martins are perfectly sexually monomorphic. However, for keel length females are slightly, but significantly, smaller than males. The young of the year are similarly sized but lighter than the adults. Adult females have a greater tendency than males to show greyish rather than pure white belly plumage. In the breeding season, the most reliable diagnostic for females is the unfeathered brood patch over belly and breast, and the small diameter of the cloacal protuberance. Adult females may begin to regrow the brood patch feathers in August, before southward migration. Among the 55 adult males, we identified two with clear bare brood patches, suggesting an emancipation of their role during incubation.

Conclusion Although temporary morphological traits related to reproduction (brood patch, cloacal protuberance) make it possible to assign sex with some confidence, even in this sexually monomorphic passerine, reliable molecular sexing assays on the basis of small blood samples remain necessary to accurately describe and analyse interesting mating system variations, including an apparent emancipatory role in some males.

Despite their enormous range and great abundance (Hagemeijer & Blair 1997), with some notable exceptions (Lind 1960, Bryant 1975a) there have been few studies on the social and sex lives of House Martins *Delichon urbicum*. The absence of distinctive visible secondary sexual characteristics has certainly not helped. Even in the hand, it is impossible to distinguish between adult males and females on the basis of size or plumage (Rheinwald & Gutscher 1969, Glutz von Blotzheim & Bauer 1985, Cramp 1988). Lind (1960) distinguished males from females by their higher pitched and more melodious calls. According to Glutz von Blotzheim & Bauer (1985), during the breeding season only females have a bare brood patch (males a

lightly feathered one, Bryant 1975b). Without further verification, this criterion was used by Hund & Prinzing (1979, 1985), Rheinwald (1975), Rheinwald *et al.* (1976), Stokke *et al.* (2005) and presumably also by Bryant (1979).

With their nucleated blood cells, birds easily yield DNA material in the form of small blood samples, and with the advent of increasingly reliable molecular techniques, it is now possible to quite easily apply the ‘gold standard’ for sex determination in birds: molecular assays for sex-related DNA-fragment sizes from the sex chromosomes (Griffiths *et al.* 1998, Fridolfsson & Ellegren 1999). Here we apply several different molecular assays to small blood samples of House Martins sampled during three successive breeding seasons from colonies in a single village in the northern

*Correspondence author. Email: theunis@nioz.nl

Netherlands. We use these samples to verify the accuracy of these various assays and to investigate the extent to which breeding-related external traits, such as the presence of a brood patch, can indeed be used to accurately assign sex. In the process, we also re-investigated the issue of sexual size monomorphy in House Martins, as well as looking for age-related differences in size and mass.

MATERIAL AND METHODS

This study was carried out in the small village of Gaast (53°01'N, 05°24'E), province of Friesland, The Netherlands, in June–August 2005–07. Using a variety of methods (mist-netting in late afternoons and early evenings before nightfall, using small fishing nets at the entrance of individual nests during daytime, and catching birds emerging at dawn after night-time paper-plugging of individual nest entrances), we captured adult and recently fledged House Martins as the breeding seasons were well underway (earliest catch date was 11 June 2007, latest was 26 August 2006).

As soon as possible, and always within 1.5 hours after capture, the birds were processed and released. The birds were first ringed with an aluminium ring around the right tarsus and weighed to the nearest 0.1 g on an electronic balance. We measured the length of the wing (maximum chord of folded wing, to the nearest millimetre) and the length of the keel (to the nearest 0.1 mm, with an easily sliding calliper, a measure of the sternum used previously by Bryant & Westerterp 1980).

Recently fledged, or at least flighted, birds, here called juveniles, were most easily identified on the basis

of the white trailing edges of their tertials (Cramp 1988). In adults, we scored the colour of the breast and belly plumage as either pure white or 'greyish' (white with a greyish wash). We also examined whether adults showed a single large bare brood patch over abdomen and pectoral girdle, or whether this area had a light cover of downy feathers. In adults we also measured the diameter of the base of the cloacal protuberance (with a calliper, lightly squeezing the protuberance until the cloacal opening started to widen), with a lower cut-off point of 2 mm for birds hardly expressing such a protuberance. All measurements and assessments were done by a single observer (T.P.). A droplet of blood was collected from the brachial vein after a small puncture with a sterilized needle. The blood was drawn with a heparinized microcapillary tube, the puncture closed with a piece of cotton wool, and the blood stored in 96% alcohol at –20°C before DNA extraction.

DNA was extracted in the laboratory using the chelex extraction method of Walsh *et al.* (1991). Birds were sexed following Griffiths *et al.* (1998): PCR amplification of a part of the CHD gene that is located on the sex chromosomes. PCR products were separated on 3% agarose gels with males showing one band and females two bands (Fig. 1). The cases of two assigned males with clear bare brood patches (see below) led us to verify our results, as recommended by Dawson *et al.* (2001), with the alternative method of Fridolfsson & Ellegren (1999), which consists of amplifying a different fragment of the same gene. We also applied the method of Kahn *et al.* (1998), which targets a DNA section within that fragment, amplified by the P2/P8 primers of Griffiths *et al.* (1998). All these PCR products were also separated on a 3% agarose gel, with males again showing one band

Figure 1. Picture of an agarose gel (3%) showing PCR products for two females (1, 3), two males (2, 4) and the two males with a brood patch (5, 6) using the molecular sexing methods of (left) Kahn *et al.* (1998), (middle) Griffiths *et al.* (1998) and (right) Fridolfsson & Ellegren (1999). M = DNA size marker (lowest band = 100 bp, band-size increment is 100 bp per band, highest band = 1000 bp).

and females two bands for the method of Fridolfsson & Ellegren (1999). For the method of Kahn *et al.* (1998), single bands were observed in females that were higher on the gel and thicker than the bands of males (Fig. 1 left). Molecular sex assignment was consistent between all three methods for females, males and males with a brood patch (Fig. 1). Note that in ten recaptured birds (three females and seven males) blood samples were also taken in both 2006 and 2007 and similar analyses showed consistent molecular sex assignments between years.

Visual inspection of the data distributions, and the close correspondence between medians and means, showed that for the different age and sex categories the biometric data were normally distributed. To evaluate differences between categories, generalized linear models (analyses of variance) were run. All statistics were made using SYSTAT 10.

RESULTS

Bryant (1975b), who scored the degree of feathering to bareness of the brood patch on a scale of 1–5, found no feathered brood patches in incubating females and a single bare patched individual among about 70 incubating ‘males’ (note the possibility for circular reasoning in absence of molecular sexing). In our sample of 49 adults without a brood patch, 48 were indeed molecularly confirmed to be males. Rheinwald *et al.* (1976) noted that females without nests might be without a brood patch and our single exception was a female captured on 26 August 2006, the latest catch date. Bryant (1975b) showed that late in the season, females (and males) may refeather their brood patches. For our population this seems confirmed by a somewhat aberrant molecularly confirmed female that fell out of a nest entangled with a juvenile on 27 August 2007 (T. Piersma unpubl. data) and was not part of the present sample. This female showed refeathering of breast and belly.

Of 59 adult House Martins with a bare brood patch, 57 were molecularly confirmed as females and two as males. In comparison to the whole sample, neither of these two males were outliers with respect to size or coloration, timing of breeding, position of the nest (isolated or not), or the likelihood that they would have had to incubate alone because their females went missing.

Although we could statistically confirm the statements in Cramp (1988) and Turner (2004) that the underparts of adult females are usually duller than those of males, i.e. ‘greyish’ (Table 1), the difference between

Table 1. Percentage occurrence of greyish and white bellies in female and male adult House Martins captured during the breeding season and molecularly assayed for sex.

	Females (n = 58)	Males (n = 50)
Greyish belly (n = 55)	64%	51%
White belly (n = 53)	36%	49%

Based on this contingency table, the distribution of greyish and white underparts is significantly different between the sexes (Pearson $\chi^2 = 8.3$, df = 1, $P = 0.004$).

females and males in belly colour is only slight: half of the males had plain white underparts, and one-third of the females. With two exceptions, the diameter of the cloacal protuberance of adult female House Martins measured 2–3 mm (the two exceptions measuring 3.3 and 3.7 mm), whereas the cloacal diameter of 27 males always measured over 3 mm (with a maximum of 5.5 mm). With the sample sizes used, in neither males nor females did cloacal protuberance vary with time of year (ANOVA with factor month, no interactions between sex and month). Size of the cloacal protuberance during the breeding season is thus a much stronger sexual characteristic than belly plumage colour.

With average wing lengths of the four sex/age categories (Table 2) not deviating more than 0.12 mm from the overall average of 111.06 mm, House Martins certainly can be confirmed to be sexually monomorphic (Table 3). Nevertheless, average keel length of both juvenile and adult females was about 0.4 mm shorter than that of similarly aged males (Table 2) and this difference was significant, with no interactions between age and sex (Table 3). Females and males weighed about the same, but juveniles were 1–2 g lighter than adults (Table 2). There was a tendency for females to show a greater body mass difference than males between juveniles and adults (Table 2), but with the present sample sizes this interaction failed to reach significance at the 5% level (Table 3).

DISCUSSION

On the basis of several types of verifications (use of different assays, repeatability of assignments between years) we have no doubt that the DNA assay of Griffiths *et al.* (1998) is an unambiguous method for molecular sexing of House Martins. We can, therefore, indeed use this method as a ‘gold standard’ for sex identification in this species.

We could confirm the reported absence of sexual dimorphism in wing length (Glutz von Blotzheim &

Table 2. Age- and sex-related variation in wing length, keel length and body mass of House Martins captured during the breeding season.

Biometric	Statistic	Juveniles		Adults	
		Females (<i>n</i> = 20)	Males (<i>n</i> = 25)	Females (<i>n</i> = 60)	Males (<i>n</i> = 55)
Wing length (mm)	Average	111.0	111.0	110.9	111.2
	SD	3.0	3.0	3.3	2.7
	Range	106–117	106–118	105–119	105–118
Keel length (mm)	Average	18.92	19.36	19.14	19.43
	SD	0.80	0.66	0.64	0.83
	Range	17.5–20.0	17.9–20.8	17.4–20.5	17.8–21.9
Body mass (g)	Average	16.51	17.21	18.41	18.22
	SD	1.58	1.81	1.59	1.24
	Range	12.8–19.5	13.3–20.0	14.9–22.0	15.1–21.0

An analysis of variance for the different biometrics is given in Table 3.

Table 3. Analysis of variance of wing length, keel length and body mass of House Martins captured during the breeding season in The Netherlands in relation to age (young of the year = juvenile, or older = adult) and sex.

Biometric	Independent variables	df	Sum of squares	F-ratio	P
Wing length ($r^2 = 0.002$)	Sex	1	0.6	0.06	0.800
	Age	1	0.2	0.03	0.874
	Sex * Age	1	0.6	0.06	0.800
	Error	156	1398.2		
Keel length ($r^2 = 0.056$)	Sex	1	4.3	8.07	0.005
	Age	1	0.7	1.24	0.268
	Sex * Age	1	0.2	0.39	0.534
	Error	156	84.0		
Body mass ($r^2 = 0.167$)	Sex	1	2.1	0.93	0.337
	Age	1	67.9	29.65	<0.001
	Sex * Age	1	6.2	2.71	0.102
	Error	156	357.4		

Items that show significant variation between categories are presented in bold; r^2 gives the explained variance of the model. Averages, SD and ranges of the different biometrics are given in Table 2. Post hoc Scheffé tests showed that none of the age- and sex-specific averages for wing and keel length were significantly different from each other at the 5% level, but that for body mass there were differences between juvenile and adult males ($P = 0.056$), between juvenile and adult females ($P < 0.001$), between juvenile females and adult males ($P < 0.001$) and between juvenile males and adult females ($P = 0.013$). In addition, there were differences between the sexes in keel length ($P = 0.006$) and we could confirm the age difference in body mass ($P < 0.001$).

Bauer 1985, Cramp 1988), but discovered that females had significantly shorter keels than males. As the keel represents an important skeletal dimension (Piersma 1984, Piersma *et al.* 1984), this contrast suggests dimensions of sexual size dimorphism not reflected by wing size. The absence of wing and keel length differences between recently fledged juveniles and adults suggests that upon taking flight, House Martins have reached their final structural size, although their lower weights indicate that body stores still need augmentation before they leave the colony on southward migration.

In contrast to plumage colour and body dimensions, two phenotypic measures related to the breeding effort

(brood patch and cloacal protuberance) show clear sexual dimorphism during the breeding season. Although females were clearly and significantly distinct from males through having a bare brood patch and a small cloacal protuberance (if a protuberance at all), in a sample of 115 adults we discovered two females with rather large cloacal protuberances and two males with brood patches. Although the sexes usually share incubation duties (Lind 1960), the presence of bare brood patches (facilitating greater heat transfer to the eggs) in some males suggests that in some situations males provide greater than average paternal investments. The single female with a feathered brood patch in the sample (and evidence for a similar state in

another female), indicates that females regrow their downy belly feathers as soon as maternal duties are over.

The exceptions to the general rules for sex differentiation in House Martins described here imply that studies of the social life in this species using individually marked birds necessitate error-free molecular sexing (hence we refrained from providing discriminant functions). Without such a 'gold standard', it would be impossible to accurately describe and analyse interesting mating system variations, including an apparent emancipatory role in some males. A case in point is a colour-ringed female first captured on 8 July 2007 on the nest with her first brood. In August she was observed to simultaneously provision second-brood chicks in the same nest, and in a nest in another colony, only 25 m away but out of sight (Piersma 2008). In the absence of evidence based on a reliable sexing method, in view of the oddity of this behaviour we would probably have inferred this bird to be a polygynous male rather than a possibly polyandrous or helping female.

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